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SOME ASPECTS OF ADAPTATION IN *CHANOS CHANOS* (FORSKÅL)

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Introduction

The possibilities of culturing marine species of fishes in coastal belts containing salt or fresh-water have received much attention in recent years, following the lead given by Far Eastern countries in the culture of *Chanos*. In India, *Chanos* as well as species of mullets could be cultured on a large scale and a beginning in this direction has already been made in certain South Indian centres. A study of the mechanism of adaptation in these species is an essential prerequisite to any rational system of culture, since it will help in determining the optimal values of salinity and temperature of the environment for the various species.

Although presenting a general pattern of similarity in their osmotic relations, teleostean fishes differ considerably in the manner in which they adapt themselves to external salinity. In the adult eel, for instance, the gills do not appear to be able to extract chloride from fresh-water (Krogh, 1937) and the osmotic regulation is brought about by practically complete reabsorption of the chloride by the excretory system combined with a low permeability of the tissues to water (Keys, 1933). In *Fundulus heteroclitus*, on the other hand, certain cells in the gills have been considered responsible for salt regulation by the excretion of chloride in sea-water and the absorption of chloride from fresh-water (Copeland, 1948). These differences probably relate to the evolutionary history of the species, depending on whether the mechanisms of adaptation have been perfected for fresh or salt water. Specialisation has no doubt taken place even among groups and forms primarily belonging to the saline or fresh-water environment. But, as indicated by Krogh (1939), the functional peculiarities of adaptation in euryhaline teleosts are still not clear in spite of several studies on the problem.

The interspecific differences are very pronounced particularly as regards the lower limits at which fishes are able to extract chlorides. The ecological significance of this aspect of hypertonic regulation in fishes has been

discussed by Krogh (1939) who has pointed out the possibilities of fresh-waters being low enough in chloride content to present difficulties to certain species. Black (1951) finds that calcium is effective in diminishing the exchange of salts and water by *Fundulus heteroclitus* transferred to fresh-water. It is, therefore, quite likely that the power of absorbing chlorides and the lower limits of salt concentration are modified by the calcium content of the water. The importance of these factors in the adaptation of fishes to estuarine conditions has been discussed by Panikkar (1950).

Other aspects of the influence of ambient salinity relate to the size of the fish and the condition of the endocrine organs. The eel is relatively stenohaline between the time of hatching and entrance into the new environment (Krogh, 1939). In the stickleback, the power of osmotic regulation is significantly diminished during the breeding season (Gueylard, 1924) and sexually mature sticklebacks prefer brackish or fresh water, this effect being simulated when thyroid is administered to the fish *via* the alimentary tract (Koch and Heuts, 1942; Heuts, 1943).

In contrast to the salinity, the temperature of the environment provokes more passive responses in fishes which are poikilotherms. Here, the possibility has, however, to be taken into consideration of a combined influence of salinity and temperature such as has been indicated in the case of certain invertebrates (Broekhuysen, 1936; Panikkar, 1940; Broekema, 1942). Heuts (1945) has found that the geographical distribution of two subspecies of *Gasterosteus aculeatus* corresponds with the results on the effects of temperature on salt balance. It is obvious then that descriptions of heat death, cold death and optimal temperatures are incomplete without reference to the salinity of the medium employed.

In the present paper, observations are reported on the adaptive responses of *Chanos chanos* to variations in the salinity and temperature of the external medium and on the probable influence of calcium ions on adaptation. *Chanos* is a coastal species the fry of which are observed in large numbers in many places on the Madras coast (Ganapati *et al.*, 1950). It is usually reported euryhaline but reliable experimental or field data on the limits of salinity and temperature tolerance of the species are lacking.

MATERIAL, METHODS AND RESULTS

Blood serum chloride in Chanos *fingerlings*

Fingerlings of *Chanos* (65–140 mm. long) were acclimatised to the experimental media (tap water, sea-water diluted with tap water or sea-water to

which common salt had been added) for a minimum of 48 hours before blood samples were taken from the heart or caudal blood vessels by the use of glass cannulæ. The samples were kept in the frigidaire for the separation of serum from clot. For the determination of chloride, the method of Wigglesworth (1937) was used. In Fig. 1, the mean values of serum chloride of 3–6 specimens of *Chanos* are plotted against chloride in the external medium.

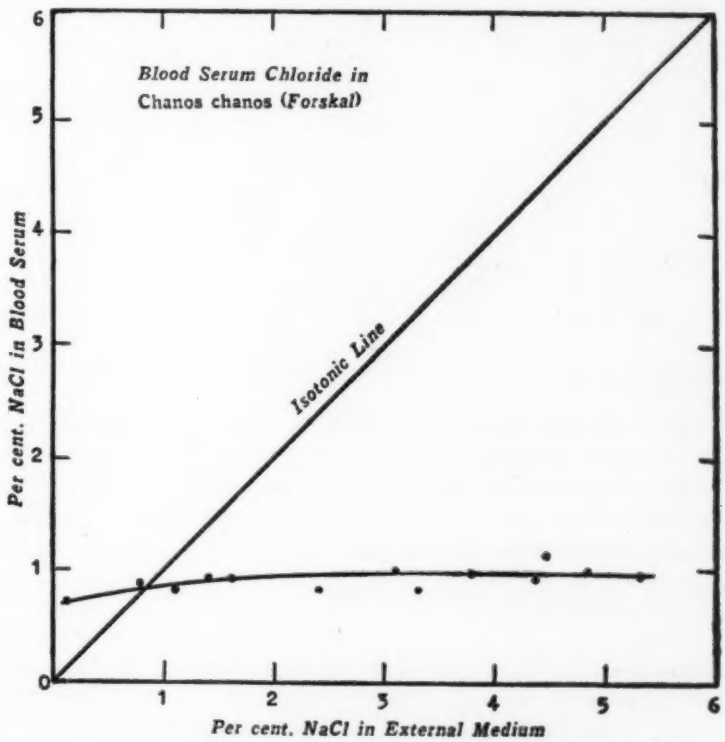

FIG. 1. Blood serum chloride in *chanos chanos* (Forskål)

Survival of Chanos *fingerlings in low salt concentrations*

In Tables I and II are given the results obtained by experiments with *Chanos* fingerlings in media containing low concentrations of salts. The external media were prepared by diluting tap water with glass-distilled water and by dissolving pure sodium chloride, calcium chloride and mixtures of these chlorides in glass-distilled water. The chloride and calcium content

TABLE I

Survival and Serum Chloride Values for Chanos *Fingerlings in Tap Water (Cl, 475 p.p.m. Ca, 112 p.p.m.) diluted with Glass-Distilled Water*

Date of experiment	% tap water in medium	Length of fish (mm.)	% NaCl in fish blood serum	Survival (hrs.)
26–12—49	100	70	0·969	*72
5–12—49	10	..	..	*24
7–12—49	1	..	..	*24
23–12—49	1	84	0·609	*72
9–12—49	0·1	..	..	*24
21–12—49	0·1	..	0·490	*24
17—1—50	0·1	123	..	19
4—4—50	0·1	..	..	*48
10–12—49	0·01	..	..	24
24—1—50	0·01	..	..	*48
19–12—49	0·008	96	0·372	*24
20–12—49	0·008	123	0·541	19
14–12—49	0·007	..	..	24
16–12—49	0·007	62	..	6
17–12—49	0·007	65	..	11
12–12—49	0·002	76	..	5
11–12—49	0·001	72	..	3

* The fish were alive at this stage but further observations were not made.

of tap water (and the fresh-waters listed in Table VII) were estimated by standard methods described in books on water analysis. Before transfer to the experimental media, the fingerlings were given a brief washing in glass-distilled water. One litre of medium was used per fingerling.

Thermal resistance of Chanos

The fish used were preacclimatised to the experimental media for a day.

(*a*) *Resistance to heat.—Chanos* fry, 10–20 in number, were kept in 1·5 l. of water contained in a glass cylinder (14 cm. ht.; 14 cm. diam.) and the temperature of the water was steadily raised by heating it in an air oven. The rate of increase of temperature was adjusted to about 4° C. per hour. The required temperature was maintained by switching the current on and off at intervals of 15 minutes. In experiments with the fingerlings, the fish was kept in 2·5 l. of water contained in a glass vessel (8″ diam.; 4″ ht.). The temperature of the water was raised over a closed type of electric hot plate (1,000 watts; 230 v.). The current was switched on initially for 1 minute and then at the rate of 1 minute for every 0·5 hr. till the temperature of water was 33° C. After this the rate of passing the current was increased to 1 minute per 15 minutes till the water attained the temperature of 35° C.

TABLE II

Survival and Serum Chloride Values for Chanos *Fingerlings in 'Artificial' Media: Figures within brackets in column 3 refer to calculated values of ionic concentrations, expressed in p.p.m.*

Expt. No.†	Date of expt.	Medium	Length of fish (mm.)	% NaCl in fish blood serum	Survival (hrs.)
(i) 1	25–11–49	Glass-distilled water	83	0·733	1·1
(i) 2	25–11–49	do	79	0·710	2·1
(ii) 3	20–10–52	do	76	..	*192
(ii) 4	20–10–52	do	97	..	*192
(ii) 5	20–10–52	do	89	..	192
(iii) 6	30–10–52	do	71	..	*24
7	16–8–50	NaCl (0·008)	87	..	1·3
8	4–12–49	do (84)	..	..	11
9	11–4–50	do (421)	..	..	*7
10	26–4–50	do (421)	..	..	*48
11	16–8–50	NaCl + $CaCl_2$ (Na, 0·003; Ca, 0·00002)	100	..	*48
12	9–2–50	do (Na, 0·177; Ca, 0·088)	..	..	5
13	4–2–50	do (Na, 0·177; Ca, 0·088)	71	..	*48
14	1–2–50	do (Na, 0·177; Ca, 0·883)	..	..	*48
15	29–11–49	$CaCl_2$ (14)	..	..	3·5
16	30–11–49	do (29)	83	..	*7
17	2–12–49	do (72)	..	..	7
(i) 18	28–11–49	do (144)	71	0·946	*48
19	28–3–50	do (144)	90	1·009	*48
20	28–3–50	do (144)	97	1·042	*48
21	28–3–50	do (144)	93	1·042	*48

* The fish were alive at this stage but further observations were not made.

† (i) Direct transfer from water of salinity 1·44% NaCl.

(ii) Fish preacclimatised for 4 months to fresh-water in a pond (salinity 1 part per 1000).

(iii) Fish preacclimatised for 4 months to fresh water and then for 42 hours to sea-water.

In experiments Nos. 7–17 and 19–21, the fish were collected from a saline pond and preacclimatised to tap water for 24 hours.

The subsequent maintenance of temperature at the required level was carried out by switching on the current for 0·5 or 0·75 minute at intervals of 15 minutes. Tables III and IV contain the results of experiments on the resistance of *Chanos* fry and fingerlings to heat.

(*b*) *Resistance to cold.*—The proportions of the number of fry (or fingerlings) and the volume of the medium were the same as in the experiments on the resistance of the fish to heat. The container was placed in a frigidaire for bringing down the temperature of the water. Tables V and VI contain the results of experiments on the resistance of *Chanos* fry and fingerlings to cold.

TABLE III

Resistance of Chanos *Fry* to Heat*

Date of expt.	Medium	No. of fry used	Temp. of water† (deg. C)		Time (Hours)										Per cent. survival of fry
			Initial	Test temp.‡	1	2	3	4	5	6	7	8	9	10	
25-4-50	Sea-water	20	30·6	39·7±0·1	100	100	90	85	..	..	..	..	..	..	
26-4-50	do	20	29·8	41·1±0·3	100	95	95	95	95	95	..	..	..	..	
27-4-50	do	20	29·6	41·6±0·2	75	65	55	55	..	50	50	50	..	..	
3-5-50	do	20	31·7	42·9±0·1	50	..	..	..	..	..	..	..	..	..	
1-5-50	do	20	30·0	42·9±0·2	60	55	40	..	25	..	20	15	5	0	
5-5-50	do	20	31·0	43·0±0·2	80	40	35	25	25	20	..	15	..	..	
3-5-50	do	20	29·6	43·1±0·4	20	0									
13-6-50	½ (sea-water)	10	29·9	39·2±0·2	100	100	100	100	100	..	..	..	..	..	
4-7-50	do	10	29·4	40·1±0·0	70	70	60	50	50	50	50	50	..	..	
5-7-50	do	10	30·0	41·4±0·4	100	80	80	70	70	70	..	..	..	..	
6-7-50	do	10	30·6	42·2±0·2	90	90	80	70	50	50	40	40	..	..	
29-5-50	Tap water	10	30·6	40·1±0·3	100	100	100	100	100	100	100	100	..	..	
30-5-50	do	10	30·0	40·8±0·4	100	100	100	100	100	..	..	..	..	..	
31-5-50	do	10	31·0	42·0±0·4	100	100	100	100	..	..	..	..	..	..	
1-6-50	do	10	29·8	43·0±0·3	10	..	..	..	..	..	..	..	..	..	

* Size of the fry used in the experiments : No. of fry whose lengths were measured 115 ; range, 13–30 mm. ; mean, 17·4 mm. ; & s.d., 3·3 mm.

† The rate of increase of temperature was about 4° C. per hour.

‡ Temperatures were recorded at intervals of 15 minutes and the figures correspond to (mean ± s.d.) of these readings.

The Analysis of variance of the data in Table III is given below:—

Source of variation	S.S.	D.F.	Variance	F by calcn.	F for P=0·05
Total ..	12124·3952	95	..	..	..
Temperatures, T ..	1161·1240	3	387·0413	4·08	2·83
Hours, H ..	2183·2593	7	311·8942	3·29	2·24
Salinities, S ..	40·2255	2	20·1127	4·72	19·5
Interaction : TS	314·4630	6	52·4105	1·81	3·8
Interaction : HS	621·2329	14	44·3738	2·14	2·3
Interaction : HT	3817·5219	21	181·7867	1·92	1·7
Residual ..	3986·5686	42	94·9183	..	..

At the P = 0·05 level, the only significant sources of variation are temperature, hour and interaction HT.

TABLE IV

Resistance of Chanos *Fingerlings to Heat*

Expt. No.	Date of expt.	Medium	Temperature of water (deg. C.)		Rate of increase (deg. per min.)	Survival (Hrs.)	Length of fish (mm.)
			Initial	Test temp.*			
1	22-12-50	Sea-water	26·2	(34–35)	8·6/191	‡6	117
2	12-12-50	do	25·8	34·6±0·3	8·9/195	3	135
3	16-12-50	do	25·7	35·2±0·1	9·5/234	‡7	..
4	5-12-50	do	28·5	(37–38)	8·2/20	‡10	..
5	8-12-50	do	25·9	(38–39)	11·6/198	‡3·3	..
6	20—7-50	do	27·9	(40·5—41·2)†	13·3/155	0·3	128
7	19-12-50	Tap water	27·6	(34·5–35)	7·6/157	‡6	133
8	13-12-50	do	26·7	35·4±0·2	8·7/195	‡3·5	..
9	6-12-50	do	28·3	(40·5—40·9)	11·3/223	§0·2	..

* In experiment Nos. 2, 3 & 8, the test temperatures correspond to (mean of temperature readings at 15 minute intervals ± s.d.). In all the other experiments, only the range of temperature has been indicated.

† By the procedure described for the fry.

‡ The fish were alive at this stage but further observations were not made.

§ The fish was in distress and jumped out of tank when the temperature was raised to 41° C.

TABLE V

Resistance of Chanos *Fry* to Cold*

Date of expt.	Medium	Temperature of water (deg. C.)			% Survival of fry
		Initial	Test temp.	Rate of fall of temp. (deg. per min.)	
12—5—50	Sea-water	29·0	21·8	7·2/390	100
15—5—50	do	30·1	18·0	12·1/525	100
13—5—50	do	28·5	13·8	14·7/600	58
		28·5	13·2	15·3/660	33
30—5—50	½ (Sea-water)	29·1	14·0	15·1/255	70
		29·1	14·0	15·1/285	50
		29·1	14·0	15·1/345	40
15—5—50	Tap water	33·2	20·7	12·5/335	93
		33·2	20·3	12·9/365	83
		33·2	18·1	15·1/485	75

* Size of fry : No. of fry whose lengths were measured ; 52.
Range ; 15-25 mm. Mean ; 19·4 mm. S.D. : 2·5 mm.

TABLE VI

Resistance of Chanos *fingerlings to cold*

Date of expt.	Medium	Temperature of water (deg. C.)			Length of fish (mm.)	Remarks
		Initial	Test temp.	Rate of fall of temp. (deg. per min.)		
22—11—50	Sea water	28·5	15·1	13·4/220	22	
30—11—50	do	25·2	12·3	12·9/190	122	Fish in distress
1—12—50	Tap water	27·6	14·4	13·2/165		
24—10—50	do	25·9	13·9	12·0/535		
17—10—50	do	25·8	13·6	12·2/285		Fish in distress
29—10—50	do	26·5	11·2	15·3/485	75	Fish comatose

DISCUSSION

For a variation in the external salinity corresponding to 5% NaCl, the change in the blood serum chloride of *Chanos* fingerlings is only of the order of 0·3% NaCl (Fig. 1). The chloride values for fingerlings kept in 1% and lower dilutions of tap water (equivalent to 5 p.p.m. of chloride and less) indicate a progressive breakdown of the osmoregulatory mechanism extending over a long period during which the loss of salt is more than what could be made good by the active uptake of ions.

The results with the 'artificial' media containing both sodium and calcium chlorides agree with those obtained with tap water of about equal ionic concentrations (Tables I and II). Since tap water differs from glass-distilled water chiefly in its content of chloride, sodium and calcium, the results obtained with the two sets of media may be taken to correspond to the effects of these ions. It is also seen that the survival of the fingerlings in glass-distilled water improves with prolonged preacclimatisation to fresh-water or by the addition of chloride, either as NaCl or $CaCl_2$, to the medium. For the same survival time, it would appear that less of either chloride suffices in a mixture than when the chlorides are used separately. Of special significance is the response of the fingerlings to solutions of calcium chloride, the mean serum chlorides (1·01% NaCl) in this case being considerably higher than what would be expected (0·7% NaCl, Fig. 1) in a medium of tap water of similar salinity. Evidently, the retention of blood chloride is facilitated by the presence of calcium in the external medium, more so in the absence of the antagonistic ion, sodium. In the light of this

protective action of calcium, statistics on the survival and growth of *Chanos* in hard and soft natural waters would afford interesting material for comparative study.

The observations on the survival of *Chanos* in low salt concentrations further suggest the necessity for a more precise definition of 'fresh waters' used in *Chanos* culture than is at present available. Fresh-waters vary widely in their content of chloride and calcium (*cf.* Table VII). The possibility has to be borne in mind that certain fresh-waters especially those in the interior (*cf.* Krogh, 1939), may contain these ions in amounts lower than the critical limits. Under these conditions, *Chanos* may be losing blood salts to the surroundings and continue to live only till the minimum threshold value for blood salts is reached. Such waters, however, would not be suitable for large-scale culture of fish.

TABLE VII

Chloride and Calcium Content of Some Natural Fresh-Waters

Fresh-water	Cl. p.p.m.	Ca p.p.m.	Date
Upputeru (opp. Jungampadu) ..	72	28	19-11-47
Kondangi ..	79	25	17-11-47
Madras (Univ. Zool. Lab. tap water) ..	..	17	5-3 -48
Mandapam tap water ..	475	112	17-12-49
Akividu well water ..	3397	361	17-11-47

The time of exposure to the test medium and the rates of increase or decrease in temperature are likely to influence the heat death and cold death temperatures (Heilbrunn, 1947). Data on these have therefore been included in Tables III–VI. On the basis of the standard time of exposure of 30 minutes suggested by Heilbrunn, the heat death temperatures of *Chanos* fry and fingerlings are about 43° C. and 39° C. respectively which are higher than those recorded for marine fishes (Heilbrunn, *loc. cit.*). The upper lethal temperatures of fishes have been correlated with habitat by Hart (1951) who finds that species from base level streams and marshes have the highest lethal temperatures. It is interesting to note that the temperature of the water in the tidal flats from which *Chanos* fry were collected was on two occasions as high as 36° C. and 38·5° C., suggesting that in the present instance the high heat death temperature is possibly the result of adaptation to the natural habitat. From the analysis of variance of the data in

Table III, it is also seen that variations in the salinity of the water are not significant in respect of the per cent. survival of the fry under conditions of heat death.

In regard to the resistance to cold, the results in Tables V and VI indicate that *Chanos* fry and fingerlings can withstand momentary exposures to 18° C. and 14° C. respectively, both in sea-water and in tap water. In the absence of facilities for the maintenance of low and steady temperatures, the cold death temperatures and the influence of salinity on these could not be studied in detail.

SUMMARY

1. A study has been made of the adaptive responses of *Chanos* to variations in external salinity and temperature. In external salinities varying from 0·1% NaCl (hard tap water) to 5·3% NaCl (sea-water *plus* common salt), *Chanos* fingerlings retain their blood serum chloride values fairly constant over a narrow range of 0·3% NaCl. In dilutions of tap water corresponding to a chloride content of 5 p.p.m. and less, the serum chloride values suggest a breakdown of the osmoregulatory mechanism.

The period of survival of *Chanos* fingerlings in glass-distilled water is prolonged if they have been preacclimatised to fresh-water or if sodium or calcium chloride has been added to the medium. The serum chloride values for fingerlings kept in solutions of calcium chloride (144 p.p.m. of Ca) indicate that calcium in the external medium aids the fish in the retention of blood salts.

The chloride and calcium contents of natural fresh-waters appear to be of critical significance when *Chanos* is transferred direct from high salinities.

2. The heat death temperatures of *Chanos* fry and fingerlings (43 and 39° C. respectively) are higher than those reported for marine fishes. The heat death temperature of the fry is not significantly influenced by the salinity of the medium, the per cent. survival of the fry at any temperature being dependent only on the temperature of the water and the time of exposure.

The fry and fingerlings are able to withstand momentary exposures to 18° C. and 14° C. respectively, both in sea-water and in tap water.

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EPIDEMIOLOGICAL ASPECTS OF THE BOMBAY DUCK OR 'BOMBIL' (*HARPODON NEHEREUS* BUCH.)

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(Communicated by Dr. T. S. Mahabale, F.A.SC.)

Introduction

Sea water or marine animals are not generally regarded as vectors of diseases. Nevertheless, the sea presents grave problems as far as public health is concerned for it is to be expected that harbour waters generally contain a large load of terrigenous bacteria. The pollution of bays and estuaries with unpurified sewage, and the insanitary conditions prevailing all along the thickly populated Indian coast, renders the sea water a potential source of infection both to the fish-eating population as well as to those bathing in these waters. The probability of *Harpodon nehereus*, and the micro-organisms associated with it, being involved in food poisoning cases has already been indicated in an earlier communication (1952). Added to this, the fish abounds in coastal waters, which is so often polluted with sewage and other fæcal matter from terrigenous sources which is likely to constitute the feed of the fish and thus indirectly constitute human food. Again, the extremely delicate constitution of this fish together with its high moisture content, does not permit a satisfactory penetration of heat during the cooking process owing to the rapidity with which it gets underdone or actually burnt. This provides an opportunity for the bacteria associated with it to outlive some of the culinary processes. The probable role of this and/or other varieties of fish in the dissemination of intestinal diseases is further emphasised by the fact that epidemics of cholera and typhoid fever have been observed to coincide with periods of the highest 'bombil catch' and that such epidemics not infrequently have their origin in the fishing villages.

Experimental

Selection of pathogens.—A large number of pathogenic bacteria are of intestinal origin and it would appear that a dozen odd species are worth trying from the point of view of their association with sea food. Actually however, from the experience gained in this laboratory with regard to similar studies on sugarcane juice (1948), *mawa* (1948), watermelon (1948), *nira* (1949) and curds (1950) in the dissemination of intestinal diseases, it has been

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revealed that it is not necessary to put to test a large number of pathogenic bacteria, and as such only five important species were employed. The species selected were: *Salmonella typhosa*, *Salmonella schöttmuelleri*, *Vibrio comma*, *Escherichia coli* var. *communis*, and *Micrococcus pyogenes* var. *aureus*. *Salmonella typhosa* has been associated with oyster and other shell-fish poisoning. Its inclusion will go undisputed, and the Felix Ty 2 strain was employed in this investigation. *Salmonella schöttmuelleri* was chosen to represent the paratyphoid and food poisoning groups. Its extreme adaptability, its incidence in different food items, and the frequency of its association with enteric fever, were other reasons which justified its choice. The choice of *Vibrio comma* was made in the light of the fact that cholera spreads very rapidly during the intermittent rainy and hot spell days in places like Bombay and other coastal towns, when the fish makes its appearance in large numbers. *Escherichia coli* var. *communis* was included as a representative of the tolerant intestinal bacteria which are an index of sewage or similar pollution. As a representative of gram-positive bacteria, we preferred to use *Micrococcus pyogenes* var. *aureus* specially because of the importance nowadays attached to staphylococcal food poisoning. All the strains used in these investigations, were of the standard type collection (N.C.T.C.—U.K.).

Experimental procedures adopted.—The survival of various human intestinal pathogens in the *bombil* is the first prerequisite to this fish acting as a carrier of intestinal diseases. Viability studies on selected species of bacteria in the fish and its immediate surround, *viz.*, sea water are therefore of prime importance. Likewise the effect of heating and other culinary procedures on the survival and proliferation of these significant microbial entities would also appear to be important from the epidemiological standpoint.

Viability studies of the pathogens in sea water were carried out with 18 to 24 hours cultures on nutrient agar (pH 7·6). The growth was very gently washed off with sterile physiological saline, and 2 opacity suspensions of the respective cultures were made, comparing it with standard opacity tubes. An 0·2 ml. aliquot of these suspensions were then transferred with the aid of sterile pipettes, into two sets of sterile tubes, one containing 9·8 ml. of sterile sea water, and the other 9·8 ml. of raw sea water, maintained for comparative purposes. Both the sets were incubated at the room temperature (28–30° C.). A definite quantity of the contents from each tube was plated on MacConkey's agar and nutrient agar initially and at regular intervals of time. The relative growth of each type of pathogen was determined after 48 hours of incubation at 37° C.

The results of viability studies in sea water led us to the possibilities of the infested fish constituting a potential danger to human health. The viability and/or proliferation of ingested pathogens in the alimentary canal or muscular tissue of the fish naturally suggested itself to be the most pertinent line of investigation. An ideal method would be to infect specially constructed tanks containing sea water and marine life including *bombil*, and after suitable intervals, examining the contents of the alimentary tract of the fish, by plating procedures. Lack of aquarium facilities, however, compelled us to resort to *in vitro* studies. The susceptibility of the *bombil* to easy decomposition, rendered such studies difficult. Nevertheless, the viability of the organisms in sterile fish tissue and incised ventriculus has been examined, and the two methods adopted by us are outlined below:—

(1) Fish tissue or incised ventriculus were sterilized in petri-dishes and in culture tubes and inoculated with 0·2 ml. of the standard suspension of the respective pathogen, the number of viable organisms initially present in the inoculum being previously determined by plating procedures. After appropriate incubation periods, the increase or decrease of the test organisms in the tissue was observed by similar methods.

(2) 2 g. of fish tissue or incised ventriculus were weighed out on a watch-glass and then transferred into an Erlenmayer flask containing 50 ml. of distilled water. Five such flasks with their contents were sterilised and each inoculated with 0·2 ml. of the 2 opacity suspension of the different pathogenic intestinal bacteria already enumerated. A loopful of the contents was plated out immediately after inoculation and subsequently at intervals of 1 hour, for 7 hours in the first instance, and subsequently after longer intervals, *viz.*, 24 hours, 48 hours, 5 days, 15 days, 20 days, 25 days, 30 days and 35 days. In the case of the intestinal pathogens, the medium used for plating purposes was MacConkey's agar; plating from the *M. pyogenes* var. *aureus* inoculated flasks was done on nutrient agar. The temperature of incubation was 37° C.

Inasmuch as fish naturally infected in sea water are sold in the market either fresh or dehydrated, this approach appeared to us reasonable, the more so as we extended our observations to dehydrated and refrigerated fish. In these latter studies, the fresh fish was artificially infected with a pathogenic bacterium, and the effect of refrigeration, holding at room temperature, and desiccation on the viability of the bacterium both on the flesh and in the intestinal tract of the *bombil* noted. Both inoculation of the fish and withdrawal of the material was accomplished with the aid of a sterile syringe fitted with a special probe. Plating was carried out on Mac Conkey's agar

and nutrient agar and a control indicated the bacteria normally present on the fish. Physical changes on the fish induced on storage, were also noted.

Inasmuch as all varieties of *bombil* are subjected to heat and other culinary procedures before consumption, the survival or otherwise of these pathogens as well as other bacteria normally present on the fish, during such procedures, would provide interesting data. Considering the delicate structure of the *bombil*, as compared to other fishes, the heat treatment had to be

TABLE I

Viability of the Test Pathogens in Raw and Sterilised Sea Water

(Survivors in percentage)

9·8 c.c. of sea water + 0·2 c.c. of 2 opacity suspension	Time of incubation in hours						Time of incubation in days				
	½	6	24	48	54	96	6	7	8	9	10
A. UNSTERILIZED SEA WATER											
Escherichia coli ..	100	55	nil	nil	nil	nil	nil	nil	nil	nil	nil
Salmonella typhosa ..	100	95	nil	nil	nil	nil	nil	nil	nil	nil	nil
Salmonella schöttmuelleri	100	100	nil	nil	nil	nil	nil	nil	nil	nil	nil
Vibrio comma ..	100	137	0·1	nil	nil	nil	nil	nil	nil	nil	nil
M. pyogenes var. *aureus*	100	130	20	*	20–30	5		nil	nil	nil	nil
B. STERILIZED SEA WATER											
Escherichia coli ..	100	95		*	0·1	0·1	nil	nil	nil	nil	nil
Salmonella typhosa ..	100	115	20	*	10	2·5	0·1	nil	nil	nil	nil
Salmonella schöttmuelleri	100	110	5	*	0·5	0·2	0·1	nil	nil	nil	nil
Vibrio comma ..	100	120	10	*	20	9	4·5	13·5	*	2	0·2
M. pyogenes var. *aureus*	100	120	100	*	50	7	*	0·2	nil	nil	nil

* Could not be ascertained.

very carefully manipulated in order to retain the physical nature of the fish without at the same time undercooking it. Prior to cooking the fish by the methods commonly employed in the Indian home, *viz.*, by roasting, grilling, boiling, steaming and frying, the fresh fish or the dried form on reconstitution was inoculated with a standard opacity suspension of the pathogenic cultures and the survival or destruction of these micro-organisms to culinary processes recorded. Although commonly the *bombil* is not consumed after roasting, steaming, or boiling, we have included also these in our experiments, together with the shallow fat frying methods. The dehydrated *bombil* is also consumed in several other forms involving the use of various ingredients, some of which are bactericidal. Since it is impossible to determine whether control of the pathogen is accomplished by heat treatment or by one or more of these ingredients, it was considered futile to attempt viability studies on such preparations. Though the fish is also used in 'curries', when consumed in this form, it is completely cooked, affording little chance of survival to the pathogens.

RESULTS AND DISCUSSION

Viability of the pathogens in sea water.—Striking differences in the viability of the test pathogens in sterile sea water and raw sea water were observed. The results (see Table I) indicate that with the exception of *Vibrio comma* all other intestinal organisms survive six days in sterile sea water, but not more than 24 hours in raw sea water. *Vibrio comma* survives in sterile sea water for ten days, but only little more than 24 hours in raw sea water. *Micrococcus pyogenes* var. *aureus* survived relatively longer in the raw sea wtaer where it persisted even after four days, whilst in sterile sea water it survived for seven days. The fact that, in general, human pathogens do not survive as long in sea water as in fresh water has long been recognized, and the experimental results of various workers have been summarised by Frankland and Frankland (1894). Our observations on the longer viability of pathogens in sterile sea water as compared to raw sea water is in agreement with the findings of many previous workers. Gelarie (1916) for instance, estimated that choleræ vibrio could be eliminated by the natural flora and other adverse conditions in New York harbour within 48 hours. ZoBell (1946) failed to find colifom bacteria in any of the 961 samples of sea water collected at stations remote from the possibilities of terrigenous contamination, though large numbers were found in polluted bays and estuaries. In his studies on the factors which influence their survival, pathogenic bacteria were found to perish very soon in the sea. The typhoid bacillus and pathogenic species of *Micrococci* were less resistant. *V. comma* lived for several days in heat sterilised sea water, but it soon

TABLE II

Viability of the test pathogens on the flesh in vitro

(Number of colonies per unit volume)

50 c.c. of distilled water 2 gm. of flesh 0·2 c.c. of 2 opacity suspension ..	Time of incubation of the flasks																
	in hours										in days						
	0	1	2	3	4	5	6	30	42	48	5	10	15	20	25	30	35
Escherichia coli ..	107	208	215	120	310	330	350	1,400	6,000	6,500	6,000	130	230	600	70	No growth	
Salmonella typhosa ..	35	68	52	79	114	160	230	3,450	6,000	6,000	9,000	2,300	750	1,300	400	No growth	
Salmonella schottmuelleri ..	362	376	303	230	400	380	550	2,750	5,030	6,000	8,000	2,800	700	1,200	230	No growth	
Vibrio comma ..	No growth							520	6,500	6,500	600	10	190	530	36	No growth	
M. pyogenes var. *aureus* ..	250	200	400	220	510	520	1,040	2,600	5,000	6,000		150	400	500	250	No growth	

TABLE III

Viability of the Test Pathogens on the Ventriculus in vitro

(Number of colonies per unit volume)

50 c.c. of distilled water 2. gm. of ventriculus 0·2 c.c. of 2 opacity suspension ..	Time of incubation of the inoculated flasks																		
	in hours												in days						
	0	1	2	3	4	5	6	7	8	24	36	48	5	10	15	20	25	30	35
Escherichia coli ..	17	22	56	91	450	600	700	900		5,000	5,500	7,000		4,500	980	620	250	64	
Salmonella typhosa ..	160	160	180	90	105	115	95	65	710	4,260		6,500		7,400	800	520	40	No growth	
Salmonella schöttmuelleri	55	130	166	185	150	180	80	110	510	4,080		6,500		7,000	800	520	40	No growth	
Vibrio comma ..					No growth					5,500		7,100		8,500	250	50		No growth	
M. pyogenes var. *aureus* ..	250						Innumerable							Innumerable					

disappeared in untreated sea water. The period of survival was a function of the organic content and bacterial pollution of water. In grossly polluted water, these organisms survived for less than 24 hours. From the results reported by other workers, as well as our own, it is evident that the possibility of sea water harbouring human pathogens cannot be altogether eliminated.

Viability of the pathogens in the bombil.—The results of viability studies in sea water have already indicated the survival of all the test pathogens, atleast for a few days, in sea water. If during this period, they are ingested by the *bombil*, this fish would constitute a potential danger to human health, provided of course, that the organisms survive and proliferate in the alimentary tract and muscular tissue of the fish.

The result of our *in vitro* studies (see Tables II and III) indicate that the pathogens under consideration, survive for more than 25 days on sterilised tissue (flesh) of the fish, when artificially inoculated with 0·2 ml. of a 2 opacity suspension of the different pathogens. Similar experiments conducted with the incised ventriculus, indicated that all the pathogens survived for 20 days whilst *E. coli* resisted the environment for even 30 days.

Experimental inoculation of young cultures of the pathogens directly into the stomach by a special syringe, and then incubating the samples under refrigeration, room temperature, and desiccated conditions, have revealed interesting results. Platings of the ventricular material at periodic intervals on MacConkey's and nutrient agar media, lead us to suggest that in the case of the refrigerated samples, except for the pathogen inoculated into the sample, no other organism including those normally present in the fish, make their appearance on MacConkey's agar. The results of the refrigerated fish inoculated with *Micrococcus pyogenes* var. *aureus* were less suggestive, owing to the fact that innumerable organisms grow on the non-selective nutrient agar, overlapping colonies of the inoculated bacterium. In the case of inoculated and dehydrated *bombils*, the normal flora of the ventriculus progressively diminishes as the dehydration progresses, the flora completely vanishing when the moisture content approximates 20%. A similar decrease in numbers is observed with the inoculated pathogens. Nevertheless, these pathogens continue to exist in small numbers even after 48 hours at the same level of moisture. With prolonged treatment in the sun, the bacterial count, as expected, progressively diminishes. At laboratory temperature and under atmospheric conditions, spoilage results very quickly and as a consequence of this, both in the inoculated as well as the control fish, the intestinal and stomach contents teem with myriads of bacteria. This indicates that spoilage may not necessarily be due to the inoculated pathogen,

colonies of which are outnumbered by other bacteria, making a correct bacterial count impossible. It was here that our *in vitro* viability studies proved valuable and the results of these are presented in Tables II and III. These indicate that the rate of growth of the different organisms is different and that whereas all the inoculated pathogens show a gradual increase in count with time of incubation, *Vibrio comma* has a long lag period of a little more than 8 hours. The relative growth phases of the individual pathogens appear in the tables.

Survival of the pathogens to culinary processes.—The possibilities of food infection may be considered to be due to either the pathogenic organisms ingested by the fish, or due to its normal microflora which may be potentially pathogenic. The survival or otherwise of inoculated pathogens as well as the bacteria normally present in the fish to various culinary processes usually employed with regard to this fish in the kitchen, provide interesting data.

The effect of heat on any particular food depends partly on the type of heat used (moist or dry), pH, time, conductivity of the food, and other factors. Moist heat is applied to food in the form of boiling, simmering, poaching, stewing, and braising. Dried heat is employed in baking, roasting, grilling, toasting and frying.

Of the different methods studied, roasting was quite effective in the destruction of all the inoculated pathogens except *Micrococcus pyogenes* var. *aureus* which makes its appearance after 7 hours incubation of the cooked sample. Grilling of the inoculated dry whole Bombay duck, as well as the laminated dry Bombay duck, has shown the presence of *Salmonella typhosa* as against the dehydrated laminated variety similarly treated. On steaming the fresh *bombil*, it was observed that the inoculated pathogens survived the treatment for five minutes, making their appearance after about 7 hours incubation. Frying procedures suggest that *Salmonella typhosa* and *Escherichia coli* could survive the treatment when fresh *bombils* were coated with bread crumb or potato paste, as is usually done before frying. The dehydrated *bombil* on dehydration, when subjected to the same treatment, indicated the complete absence of the inoculated pathogenic bacteria on about 7 hours incubation of the cooked samples. On longer incubation (24 hours), however, *E. coli* could be detected. From all this it may be concluded that *bombil* infested with any of the intestinal pathogenic bacteria would constitute grave danger to human health.

SUMMARY

The survival of the organisms *Micrococcus pyogenes* var. *aureus*, *Escherichia coli*, *Salmonella typhosa*, *Salmonella schöttmuelleri* and *Vibrio*

comma in sea water and in the *bombil* tissue has been studied. In addition the survival of these organisms in inoculated fish subsequently refrigerated, dehydrated, or held at room temperature has been studied. The effect of heat and culinary treatment on the inoculated fish has also been observed.

Our results when correlated with the casual occurrence of bacteria of fæcal origin on the flesh and ventriculus of the fish, together with the findings that cocci are present in large numbers on the skin and in almost pure culture in the ventriculus of the dehydrated fish (these have been reported in a previous communication, 1953), suggest the possibilities of this fish being a carrier of enteric infections. The fact that *E. coli*, *S. typhosa* and *M. pyogenes* var. *aureus* resist culinary treatment, also indicates that infection is possible on consuming contaminated foods, even after the cooking process.

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STUDIES ON MACKEREL FAT VARIATIONS : CORRELATION OF PLANKTON FAT WITH FAT OF FISH*

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Introduction

In a previous communication on this subject (Chidambaram *et al.*, 1952) it was brought out that the mackerels of this coast (*Rastrelliger kanagurta* Ruppel) are fatty during October-November and again during March-April and it was stated that the fatty condition of the fish was due to the intense feeding by the fish. Wimpenny (1929) has indicated that the fat content of the food of the Herring fish (a plankton feeder) has a relationship to the fatty condition of the fish, and he has described a method of estimation of fat in plankton (1938). It was decided that the fat in the surface hauls of the plankton should be evaluated and correlated with the fat in the muscle of the mackerel fish and also in the whole fish. Accordingly estimations of fat in the plankton were made and the period of study covers from January, 1950 to May, 1951.

Material and Methods

Plankton hauls were made off the coast by means of a bolting silk net and the haul was filtered. The filtrate was dried at 60° C. and the fine powder was scraped off and weighed into a thimble and extracted with ethyl ether in a soxhlet extraction apparatus. Fat on the flesh and the whole mackerel fish were estimated as indicated in a previous communication (Venkataraman and Chari, 1950).

Table below gives the average plankton fat content on a dry basis and the percentage of fat in the flesh and in the whole mackerels on a fresh or water basis.

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TABLE I

Percentage of Fat in the Flesh and Whole Mackerel Fish, and in the Plankton

	On Fresh Basis		On Moisture-free Basis
	Percentage of fat in the flesh of mackerels	Percentage of fat in the whole mackerel	Percentage of fat in the plankton
1950			
January ..	3·82	6·47	2·32
February ..	2·24	4·41	27·16
March ..	4·85	5·94	15·63
April ..	3·39	5·08	9·45
May ..	1·69	3·55	11·02
June ..	2·97	4·61	7·48
July ..	0·97	1·17	2·75
August ..	3·25	4·36	7·89
September ..	5·18	7·91	3·71
October ..	6·3	8·15	6·38
November ..	5·08	5·59	18·09
December ..	5·99	5·8	11·79
1951			
January ..	5·87	6·44	6·37
February ..	2·22	4·82	7·96
March ..	4·43	5·82	10·86
April ..	5·03	5·44	8·23
May ..	3·92	5·24	27·64
June ..	..	..	3·82

DISCUSSION

A survey of Table I and Fig. 1 will show that there is a definite relationship between the fat values of the flesh of the mackerels and in the whole fish. Again the maximum peak fat in the case of the flesh is attained durin g October-November and a small minor fatty condition during March-April. The same is the case with the whole fish analysis. This confirms our previous conclusions. The fat of plankton fluctuates rapidly and is observed to be at a maximum during February, 1950 and in May, 1951. During November

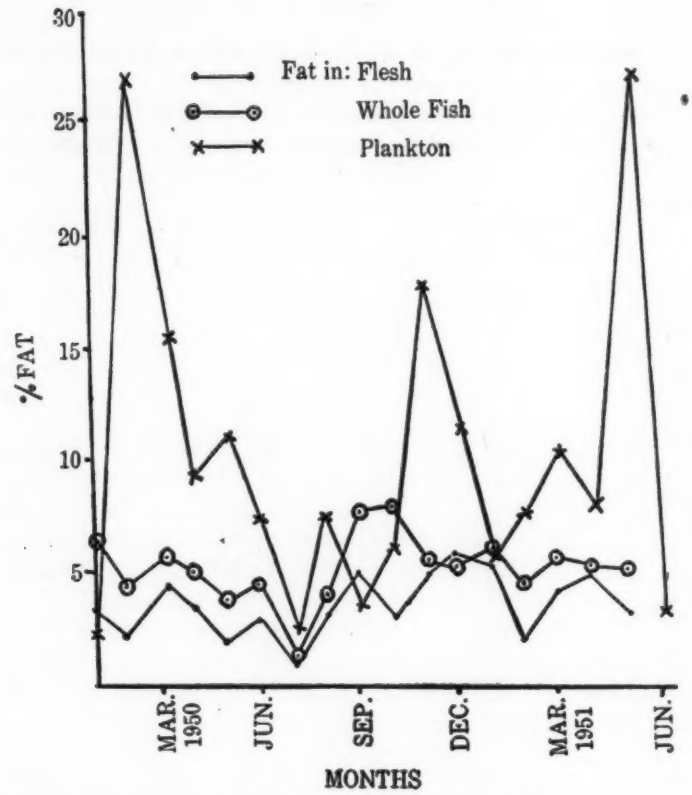

FIG. 1. Relationship of plankton fat with fat in whole, and flesh of the mackerels.

to December of 1950 and during March, 1951, the fat content is above 10% and this may well have contributed to the fat of the fish by virtue of their intense feeding as observed by examination of the volume of stomach contents (Chidambaram *et al.*, *loc. cit.*). That the fish feeds intensively during October-November and again during March-April is one proof of the fatty nature of the fish and the plankton at this time of the year is fatty enough so as to be correlated with the fattiness in the fish. The peak of plankton fat does not coincide with the peaks of the fat in the flesh and in the whole fish, and at this stage of our experiments, we are not in a position to explain this phenomenon. However, it may be indicated that this may be due to the slackness in the feeding activity of the fish or in the selectivity of the planktonic organism by the fish.

Conclusion

The average fat values for the flesh of mackerels and in the whole fish show that the fish is fatty during October-November and during March-April. The plankton can be considered to be quite fatty during March and November though the peak is attained during February and May. The fattiness of the fish during March-April and October-November can be attributed to its intense feeding on plankton rich in fat.

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ON THE NATURE OF THE SPORE-APPENDAGE IN *NEOTTIOSPORA* DESM.

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In the course of studies on Sphæropsidales from Madras a pycnidial fungus was recently collected on dead culms of *Aristida setacea* Retz. (Gramineæ) (Herb. M.U.B.L. No. 631). The fungus was characterised by the production of appendaged hyalospores, the appendage being apical. The nature of the appendage in this fungus was peculiar in that it was evanescent and easily dissolved when mounted in lactic acid or lactophenol, but could be clearly seen if mounted in water. It could be stained well with aqueous methylene blue. It seemed to us, therefore, that the appendage may be of a mucoid or gelatinous nature. This suggestion was further confirmed by an examination of the fungus mounted in India ink as suggested by Ramanathan (1938). The spores along with their appendages then appeared clearly against the dark background of India ink. It was also seen that the appendage was very thin, hyaline and like an inverted hollow cone. So far as we are aware, amongst the various genera of the Sphæropsidales-Hyalosporæ no genus has definitely been stated to have the type of appendage characteristic of our fungus (Clements & Shear, 1931). There is, however, a suggestion by Grove (1935, p. 134) that the spores of *Neottiospora* Desm. are " provided at the apex with a tuft of little mucoid setæ, which readily disappear ". Desmazieres, the author of the genus, himself (Desmazieres, 1843) did not suggest the appendages being mucoid. His description of the spores of *N. caricum*, the type species (Desmazieres, 1843, p. 347), was: " Le caractère essentiel des ces sporidies est de présenter, à l'une des extrémitiés, trois ou quatre filamens d'une tenuité extrême, simples ou bifurqués, divergens, quelquefois même un peu recourbés, et moitié environ moins longs qu'elles ". Various other mycologists have used the terms " hyaline threads " (Berkeley and Broome, 1850; Cooke, 1871), " crest " (Cooke, 1871), " penicillato-setulosæ " (Saccardo, 1884), " borstenformige..... Haare " (Lindau, 1900; Migula, 1921), " pinselformigen Borsten " (Allescher, 1901) and " Pinsel von.....Borsten " (Migula, 1921) to describe the appendages. These authors have also indicated that the spore has more than one apical appendage, but none of them have suggested that these

appendages are mucoid although most of them observed that the appendages were evanescent. The illustrations of the spores given by Cooke (1871) and by Grove (1935) are similar and depict the appendages as short, distinct bristles. The observation of Grove (1935) regarding the mucoid nature of the appendages in *Neottiospora* coupled with the observations of many authors about the evanescent nature of the appendages and also our own observations on the fungus collected at Madras, indicated the need to restudy the nature of the appendage in the genus *Neottiospora* and to find out if our own fungus could not be congeneric with *N. caricum* Desm.

We have been able to procure the type collection of Roberge on which Desmazieres (1843) based his description of *Neottiospora caricum*. This collection is in good condition and an examination of a pycnidium showed a large number of spores. The spores are hyaline, fusiform and agree with the description given by Desmazieres (1843); the appendage, however, is mucoid and in the form of an inverted hollow cone as in the case of the pycnidial fungus collected by us at Madras. The appendage may not be clearly seen in unstained preparations, but careful observation, especially after staining in aqueous methylene blue, clearly indicated the mucoid nature of the appendage in this over 110-year old dry herbarium material.

A study of the fungus collected by us indicates that the appendage is probably formed in the following way. During the development of the spore, the outermost wall (or layer of the wall ?), being inelastic, splits transversely along a line just below the middle of the spore due to the elongation of the body of the spore. The spore itself then gets detached from the conidiophore and the upper portion of the outer wall which has split is now seen as a cap enclosing the spore down to more than half its length. This thin cap later gets everted and this then appears as a hyaline, inverted hollow cone (Fig. 1) with very thin walls. It is obvious that the outer side of the cap which originally enclosed part of the spore now forms the inner side of the hollow cone. Careful examination of the fungus has shown that the lower part of the outer wall (of the spore) which has split remains attached to the conidiophore. An examination of the type collection of *Neottiospora caricum* has shown that the nature of the appendage in the case of the spores in this fungus is exactly similar to what has been described by us for our fungus. Our fungus is, therefore, congeneric with *N. caricum* Desm. The detailed description of our fungus will be given elsewhere, along with critical notes on species of the genus *Neottiospora*.

The main purport of this paper is to present the results of a study on the nature of the appendage in *Neottiospora* Desm. As a result of careful

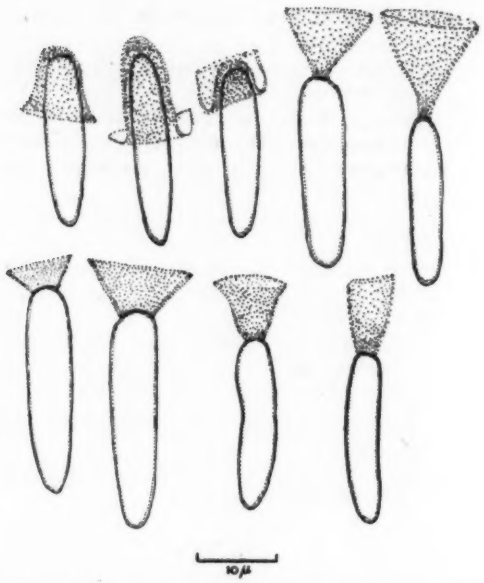

FIG. 1. Stages in the Development of the Spore-Appendage in *Neottiospora* sp. (Herb. M.U.B.L. No. 631).

study of the type collection of *N. caricum* Desm. (the type species), it may be stated that in *Neottiospora*

1. there is *only one* appendage for the spore, and it is apical;
2. the appendage is mucoid and evanescent; and
3. the appendage is in the form of an inverted hollow cone with hyaline, thin walls.

We are grateful to Prof. T. S. Sadasivan for critically reading this paper; to Prof. M. O. P. Iyengar for a most useful and interesting discussion on the nature of the spore-appendage in our fungus; to the Director, Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle, Paris, for kindly lending for study the type collection of *Neottiospora caricum* Desm.; and to Dr. V. S. Krishnan for a translation of Desmazieres' description of *Neottiospora*.

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CONTRIBUTIONS TO OUR KNOWLEDGE OF INDIAN ALGÆ

I. Chrysophyceæ—On the Occurrence of *Mallomonas* Perty, *Synura* Ehr., and *Dinobryon* Ehr. in India*

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Introduction

During a survey of the plankton algæ of inland fishery waters of North East and South India extending over a period of four years, the author has come across a number of interesting phytoflagellates. Out of these, ten species belonging to three genera of the Class Chrysophyceæ, are described in the present paper.

Most of the Chrysophyceæ, which consist of a large number of genera and species, are distributed in the temperate zone. The majority of them are known from Europe (Lemmermann, 1910; Pascher, 1913; West and Fritsch, 1927; Conrad, 1933; Fritsch, 1935; and Huber-Pestalozzi, 1941), but, some occur in North America (Smith, 1933), a few in Australia (West, 1909–11; Playfair, 1918 and 1921), Africa, North Asia and Java (Bernard, 1908; Huber-Pestalozzi, 1941) and one form in Ceylon (Lemmermann, 1907). In India, the records of Chrysophyceæ are confined to two forms of *Hydrurus fœtidus* Ag. from a cold stream in Kashmir at an altitude of 9,000 feet (Bharadwaja, 1936), *Chrysodictyon indicum* Ramanathan from a stream near Madras City (Ramanathan, 1946), and *Synura uvella* Ehr. from a pond in Madras City (Philipose, 1940).

Occurrence of the Forms

Species belonging to the genera *Mallomonas*, *Synura* and *Dinobryon* were found in fairly large numbers in widely separated places in India.

As may be seen in the table, the distribution of examples collected is spread over Assam, Bengal, Orissa, Madras and Travancore-Cochin. All the forms collected from the high elevations, *viz.*, Shillong (Approx. 4,800 ft.)

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TABLE I

Showing the Places and Periods of Collection in India of Ten Species of Chrysophyceæ with their Approximate Frequencies

Ser. No.	Name of alga	Place of collection	Period of collection	Approximate frequency
1	*Mallomonas splendens* (G. S. West) Playfair	Chandrakona (Bengal)	Dec., 1952	Rare
		Coonoor (Madras)	May–June, 1952	Isolated
		Azhicode (Tr.-Cochin)	Oct., 1951	Isolated
2	*Mallomonas tonsurata* Teiling	Chaudwar (Orissa)	Jan., 1953	Common
		Madras City (Madras)	Nov.–Dec., 1938	Isolated
3	*Mallomonas* sp.	Azhicode (Tr.-Cochin)	Oct., 1951	Rare
4	*Mallomonas mirabilis* Conrad	Kausalya Ganga (Orissa)	Nov., 1952	Common
		Sibsagar (Assam)	June, 1951	Rare
5	*Synura uvella* Ehr.	Sibsagar (Assam)	June, 1951	Isolated
		Cuttack (Orissa)	Nov., 1952 to Feb., 1953	Rare to common
		Chaudwar (Orissa)	Jan., 1953	Isolated
		Azhicode (Tr.-Cochin)	Feb., 1950 and Oct., 1951	Isolated
6	*Synura* sp.	Sibsagar (Assam)	June, 1951	Rare
7	*Synura Adamsii* G. M. Smith f. *malabarica* f. *nov.*	Azhicode (Tr.-Cochin)	Feb., 1950 and Oct., 1951	Isolated
8	*Dinobryon sertularia* Ehr.	Chandrakona (Bengal)	Dec., 1952	Rare
		Kausalya Ganga (Orissa)	Oct.–Nov., 1952	Abundant
		Coonoor (Madras)	June, 1952	Isolated
		Azhicode (Tr.-Cochin)	Oct., 1951	Common

TABLE I—(*Contd.*)

Ser. No.	Name of alga	Place of collection	Period of collection	Approximate frequency
9	*Dinobryon cylindricum* Imhof	Shillong (Assam)	June, 1951	Common
		Azhicode (Tr.-Cochin)	Oct., 1951	Rare
10	*Dinobryon bavaricum* Imhof	Chandrakona (Bengal)	Dec., 1952	Abundant
		Chaudwar (Orissa)	Jan., 1953	Common
		Kausalya Ganga (Orissa)	Oct.-Nov., 1952	Common

and Coonoor (approx. 6,000 ft.) occurred during May-June (no winter collections were available from these places), whereas those collected from the plains usually occurred during October-February, there being only one collection (from Sibsagar, Assam Valley) in which stray individuals of three species occurred in June. In most cases collections from the plains ranged over a longer duration of the year. So, it appears that these organisms do not usually occur in the plains in seasons other than winter.

The Chrysophyceæ are usually known to occur in temperate countries in cold winter weather or in cold streams and pools of mountainous tracts (Fritsch, 1935, p. 507), in plankton, usually of large lakes of clear water (Smith, 1933, p. 164), and also in shaded pools (Philipose, 1948). The occurrence of *Chrysodictyon* in a tropical area like Madras has been attributed by Ramanathan (1946, p. 185) to ideal conditions of cold weather of December-January and of plenty of shade. In the present study also, almost all the species were collected from the plains during winter, when night temperatures were fairly low, and day temperatures moderate, and the atmosphere dry. The presence of a few forms in the higher altitudes during summer could probably be explained by the fact that the range of temperature and dry atmosphere existing in the plains during winter are reached in the higher altitudes only during the summer months. However, all-the-year round collections from these areas would seem to be necessary before generalisations on their occurrence at high altitudes could be made.

All the forms recorded here were collected from inland waters ranging from small to fairly big tanks or reservoirs. However, two species of *Dinobryon* and one of *Synura* collected from Cochin occurred at the mouth

of the Periyar River, about one mile from the sea. The river water was flowing and mostly fresh, but, the area was subject to a slight tidal influence. The species concerned were two well-known freshwater forms, *viz.*, *D. sertularia* and *D. cylindricum*, and a form of *Synura Adamsii*. They were collected from distinctly freshwater areas as well. Apart from the Coccolithophoridaceæ and many naked flagellates which occur mainly in the sea (Fritsch, 1935, p. 507), a few dendroid species of Chrysophyceæ, *e.g.*, *Dinobryon balticum* (Schutt) Lemm., are found in coastal waters. Most, if not all, of these are probably fresh water forms that can survive the change into a marine environment (Fritsch, 1935, p. 529).

Description of the Forms

Genus *Mallomonas* Perty 1851

1. *Mallomonas splendens* (G. S. West) Playfair (Text-Fig. 1, A–B).

G. I. Playfair, 1921, p. 108, Pl. 2, Fig. 3;
W. Conrad, 1933, pp. 26–27, Fig. 16;
Syn. *Lagerheimia splendens* G. S. West, 1909–11, p. 74, Pl. 6, Figs. 4–8;
J. Brunnthaler, 1915, p. 136, Fig. 129.

Cells somewhat spindle-shaped with the ends rounded and 3–3½ times as long as broad. Scales quadrate and spirally arranged (they are so hyaline that it is very often difficult to make them out clearly even in specimens dried on the slide). Setæ usually four (rarely less) at each end, stout, of a spinous nature and distinctly tuberculated at the base. Anterior setæ more or less perpendicular to the cell, with their distal ends gradually curving inwards. Posterior setæ usually longer than the anterior ones and directed backwards or at a slight angle to the cell. Chromatophore light yellow, single and parietal with lateral folds. Very often these folds give the single chromatophore the false appearance of two. Nucleus single and placed between the folds of the chromatophore at the anterior end.

Dimensions.—

Length of cells	26·3–40·5 μ
Breadth of cells	10·6–13·1 μ
Anterior. setæ	14·1–17·6 μ long
Posterior. setœ	20·5–28·2 μ „

Hab.—Planktonic in a fish pond at Azhicode during Oct. 1951; in Rallia Reservoir, Coonoor, during June 1952; and in Rangamati "bundh", Chandrakona, during December 1952.

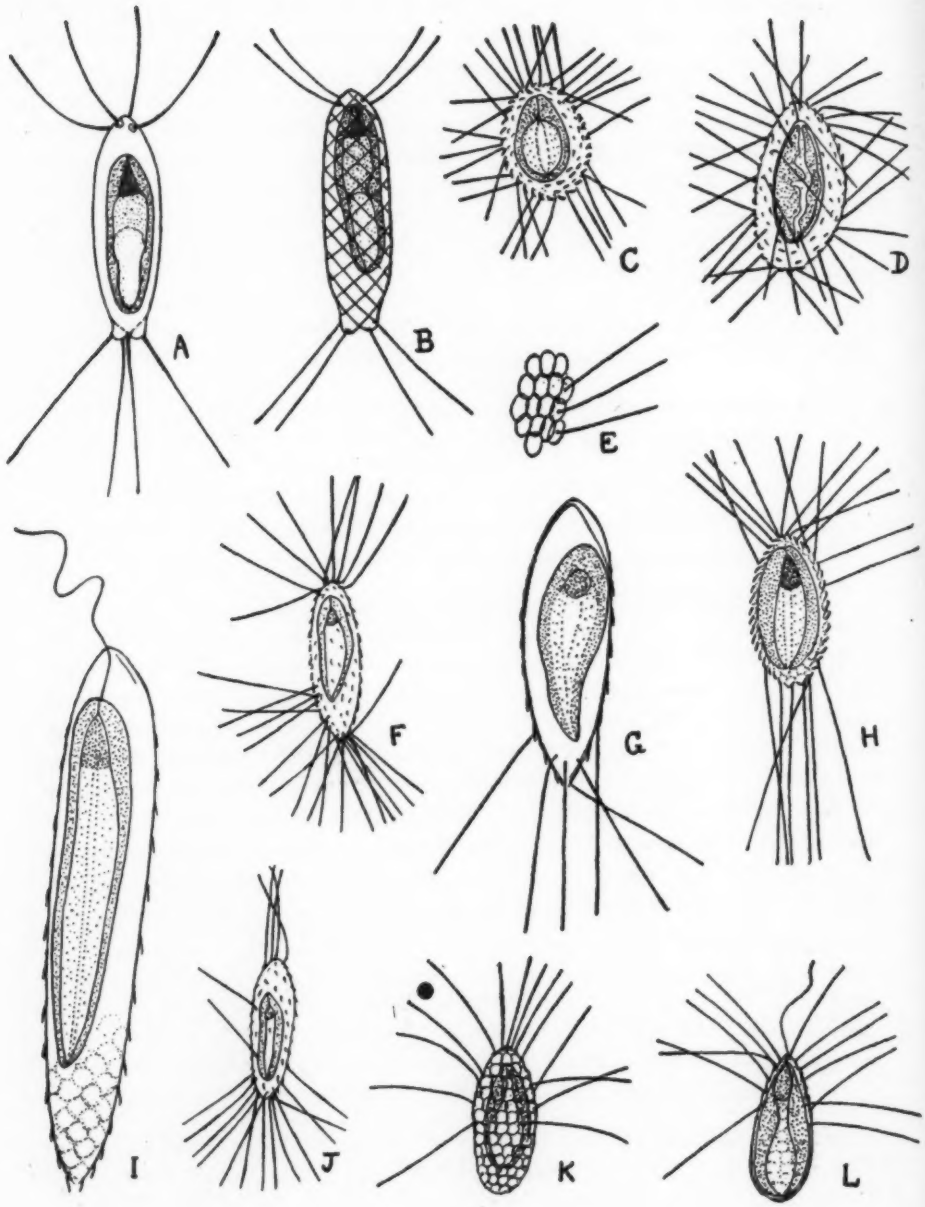


FIG. 1

TEXT-FIG. 1.—A, B, *Mallomonas splendens* (×1,000); C, D, E & H, *M. mirabilis*. E (×1,000), a portion magnified to show the scales. C, D & H (×483); F, G, I, J, *Mallomonas* sp. F & J (× 483), G & I (× 1,000); K, L, *Mallomonas tonsurata* (×1,000).

Known distr.—Australia, Holland and Java (*cf.* G. Huber-Pestalozzi, 1941, p. 100).

2. *Mallomonas tonsurata* Teiling (Text-Fig. 1, K–L).

A. Pascher, 1913, p. 38, Fig. 57;

W. Conrad, 1933, pp. 44–46, Fig. 35.

Cells ellipsoid to ovoid with the posterior end rounded and the anterior end somewhat pointed. Setæ restricted to the anterior half or two-thirds of the body. The setæ, particularly those towards the proximal end, are slightly recurved and spreading out. Scales oval and arranged in regular rows; posterior ones slightly smaller than the anterior ones. Chromatophores two in number and fairly elongated.

Dimensions.—

Length of cells	19·4–29·9 μ
Breadth of cells	8·8–10·6 μ
Setæ	17·6–21·1 μ long

Hab.—Planktonic in a fish pond at Chaudwar, Cuttack, during January 1953. Also noted from a pond in Madras City during November-December 1938.

The form agrees fairly well with the type.

Known distr. including that of var. alpina (*Pascher et Ruttner*) *Krieger.*—Europe and Java (*cf.*, G. Huber-Pestalozzi, 1941, pp. 109–10).

3. *Mallomonas* sp. (Text-Fig. 1, F–G and I–J).

Cells elongated ellipsoid, usually slightly broader at the anterior end and gradually tapering backwards. Both ends provided with varying numbers of fine setæ. Those at the anterior end are less in number (4–10) than the posterior ones (approx. 10–20). The latter are also well spread out. Scales appear to be discoid and circular and arranged somewhat spirally. They are extremely hyaline and difficult to make out even in specimens dried on the slide. Chromatophores two in number.

Dimensions.—

Length of cells	49·3–84·5 μ
Breadth of cells	14·1–16·9 μ
Setæ	28·1–35·6 μ long

Hab.—Planktonic in a fish pond at Azhicode, Cochin, during October 1951.

In its general shape and the presence of long setæ at either end, the alga comes near *Mallomonas Teilingii* (Teiling) Conrad (1933, p. 62, Fig. 54), but differs from it in having a larger number of setæ at either end, and in its being much longer (*M. Teilingii* measures 45–60 $\mu \times 10$–21 μ). In size and general appearance it resembles *M. tridentata* Nygaard (1949, p. 133, Fig. 70), which is another species with long setæ at either end, but differs from it in the setæ being much finer and larger in number. In the absence of information on the exact nature of the scales, it is not proposed to describe it as a new species.

4. *Mallomonas mirabilis* Conrad (Text-Fig. 1, C–E and H; and Text-Fig. 2, A–B).

W. Conrad, 1933, p. 67, Fig. 58.

Cells ellipsoid to ovoid with both ends somewhat rounded or acuminate, or one end slightly acuminate and the other rounded. Setæ numerous and all round the cell. Scales more or less round. Chromatophores two in number.

The alga occurred in two collections, one from nursery ponds in Kausalya Ganga, Orissa, during November 1952, and the other from a moat in Sibsagar, Assam, during June 1951. The form from Kausalya Ganga (Text-Fig. 1, C–E) measures 37·5–56·3 μ in length, 28·1–30 μ in breadth and has setæ 22·5–33·8 μ long. The alga from Sibsagar (Text-Fig. 1, H; and Text-Fig. 2, A–B) measures 40·5–60 μ in length, 18·8–22·6 μ in breadth and has setæ 26–58·1 μ long, some of the posterior setæ being distinctly longer (37·5–58·1 μ) than the rest (26–37·5 μ). In the Belgian form (*cf.* Conrad, *loc. cit.*) the setæ measure 40–55 μ in length, but, in the Indian form described here, they have a wider range of length, *viz.*, 22·5–58·1 μ.

Known distr.—Belgium (*cf.* Huber-Pestalozzi, 1941, p. 117).

Genus *Synura* Ehrenberg 1838

5. *Synura uvella* Ehr. (Text-Fig. 2, K and O).

A. Pascher, 1913, p. 50, Fig. 78;

G. M. Smith, 1920, Pt. I, p. 70, Pl. 12, Figs. 9–10;

M. T. Philipose, 1940, p. 183, Pl. 8, Fig. 163;

G. Huber-Pestalozzi, 1941, pp. 134–37, Fig. 193.

Colonies small or big, spherical or elongated; cells obovate and more or less club-shaped, short or slightly elongated and provided with forwardly

projecting short bristles. Chromatophores two in each cell. Small spherical colonies observed measured 32·4–75 μ in diameter, whereas an elongated one measured 112·5 $\mu \times 75\,\mu$. Cells ranged from 15·8–37·5 μ in length and 11·4–15 μ in breadth.

Hab.—Planktonic in fish ponds at Cuttack during November 1952 to February 1953; in a fish pond at Chaudwar, during January 1953; in fish ponds at Azhicode, during February 1950 and October 1951; and in a moat at Sibsagar, during June 1951.

Known distr.—Fairly widespread in small pieces of water in Europe (*cf.* Huber-Pestalozzi, 1941, pp. 134–35) in N. America (*cf.* G. M. Smith, 1933, p. 176); rare in Australia (*cf.* Playfair, 1921, p. 111) and in India (*cf.* Phlipose, 1940, p. 183).

6. *Synura* sp. (Text-Fig. 2, C, E–H and J).

Colony more or less spherical with cells radiating from the centre. Cells club-shaped or slightly lanceolate with a lower stalk-like portion and an expanded tip. In water mounts, clear bristles are seen only at the region of maximum width and above. When dried on the slide, the entire surface of the cell shows needle-like bristles (Fig. 2, J). Scales, from which bristles usually arise, could not be made out clearly. Chromatophores light yellow and two in each cell. Cilia two for each cell.

Dimensions.—

Diameter of colony	.. ..	97·5–105 μ
Cells: Length	.. .. ..	35·2– 59·8 μ
Maximum breadth	..	10·6– 14 μ

Hab.—Planktonic in a moat (Sibsagar Jamuna) at Sibsagar, during June 1951.

In the organisation of the colony and the structure of the cells, the present form shows a certain amount of resemblance to *Synura uvella* Ehr. on the one hand, and *S. Adamsii* G. M. Smith (*cf.* Huber-Pestalozzi, 1941, p. 144, Fig. 200) on the other. However, it does not completely agree with either species, and occupies an intermediate position between the two. Cells of *S. uvella* measure 20–40 $\mu \times 8$–17 μ and those of *S. Adamsii* 42–47 $\mu \times 6 \cdot 5$–10 μ, whereas the present form has cells 35·2–59·8 $\mu \times 10 \cdot 6$–14·1 μ. The bristles at the distal end are also more prominent. Information on the exact nature of the scales is necessary to treat it as a new species.

7. *Synura Adamsii* G. M. Smith f. *malabarica* f. *nov.* (Text-Fig. 2, D, I and L–N).

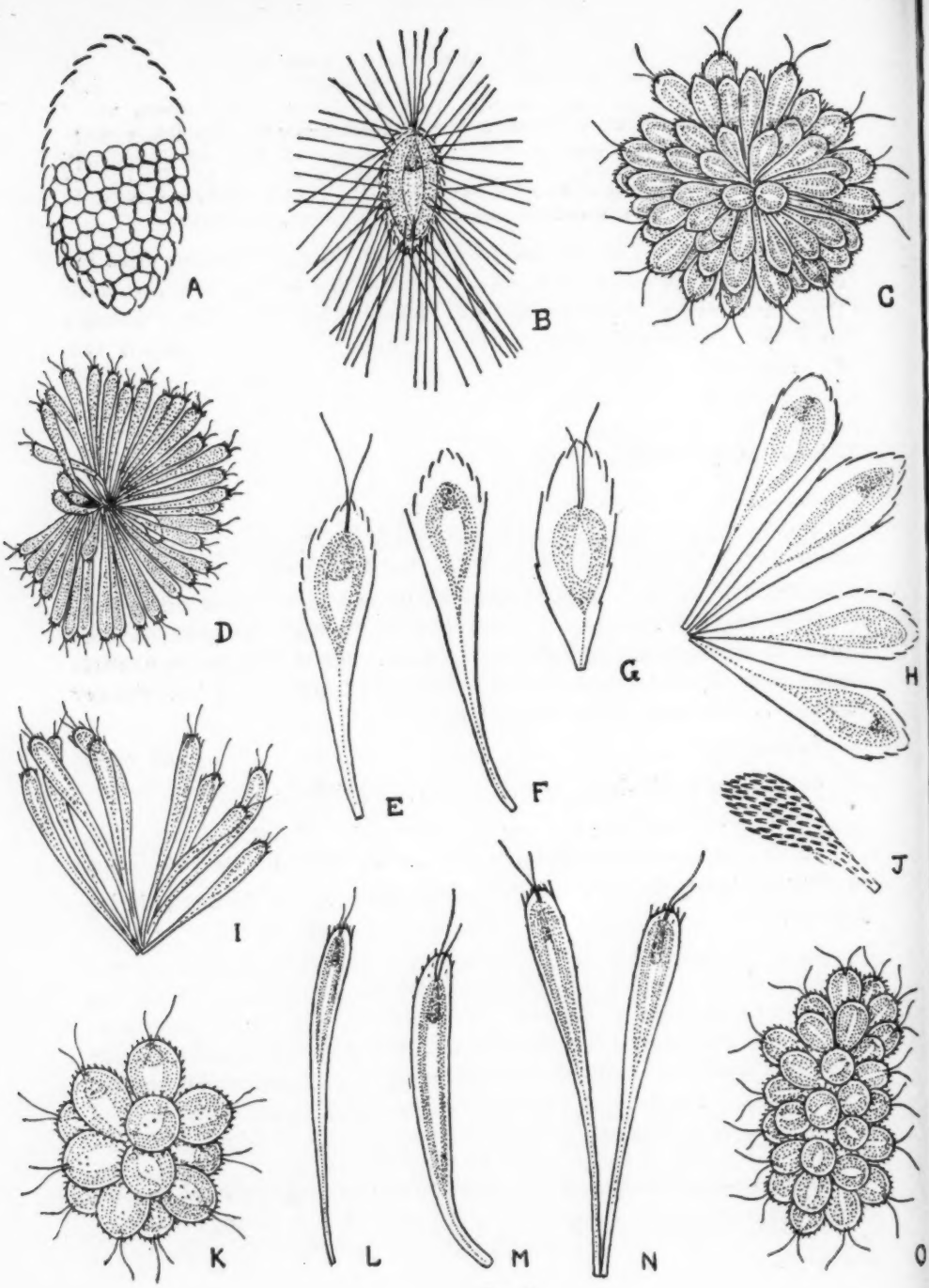


Fig. 2

TEXT-FIG. 2.—A, B, *Mallomonas mirabilis*. A (×1,000), a dried specimen showing some of the scales (semidiagrammatic). B (×483), C, E, F, G, H & J, *Synura* sp., C (× 483), a colony, E–G, individual cells. H, a group of cells. J (× 483), a cell dried on a slide to show the bristles. E–H (×1,000). D, I, L, M & N, *Synura Adamsii* f. *malabarica* f. *nov*. D (× 350), a colony. I (× 587), a portion of the colony enlarged. L–N (×1,000), individual cells. K, O, *Synura uvella*. K (×1,000), a spherical colony. O (× 483); an elongated colony.

Colony more or less loosely spherical with the cells radiating from the centre. Cells elongated, club-shaped. A few bristles are seen clearly in the apical region in water mounts. When dried on the slide, the cells show fine needle-like bristles all over. Chromatophores and flagella two for each cell.

Dimensions.—

Length of cells	..	..	54·6–75 μ
Breadth	..	..	5·3– 7·5 μ

Hab.—Planktonic in fish ponds at Azhicode, Cochin, during February 1950, and in the Periyar estuary, Azhicode, during October 1951.

The alga agrees with *S. Adamsii* G. M. Smith (*cf.* Huber-Pestalozzi, 1941, p. 144, Fig. 200; G. Nygaard, 1949, p. 139, Fig. 72, *a–c*) in most respects, but the cells were much longer and narrower than in the type, which has cells 42–47 μ long and 6·5–10 μ broad. So, it appears to be a new form and may be named f. *malabarica*, f. *nov.*

Diagnosis.—Characters mainly as in type, but the cells are much longer. Lower portion of the cell in the form of a stalk. Cells 54·6–75 μ × 5·3–7·5 μ.

Known distr. of S. Adamsii *G. M. Smith.*—N. America and Europe (*cf.* Huber-Pestalozzi, 1941, p. 144).

Genus *Dinobryon* Ehrenberg 1835

8. *Dinobryon sertularia* Ehr. (Text-Fig. 3)

Ch. Bernard, 1908, p. 209, Fig. 570;

A. Pascher, 1913, p. 72, Figs. 112 and 113;

G. M. Smith, 1920, Pt. I, p. 74, Pl. 13, Fig. 13 and Pl. 14, Fig. 1;

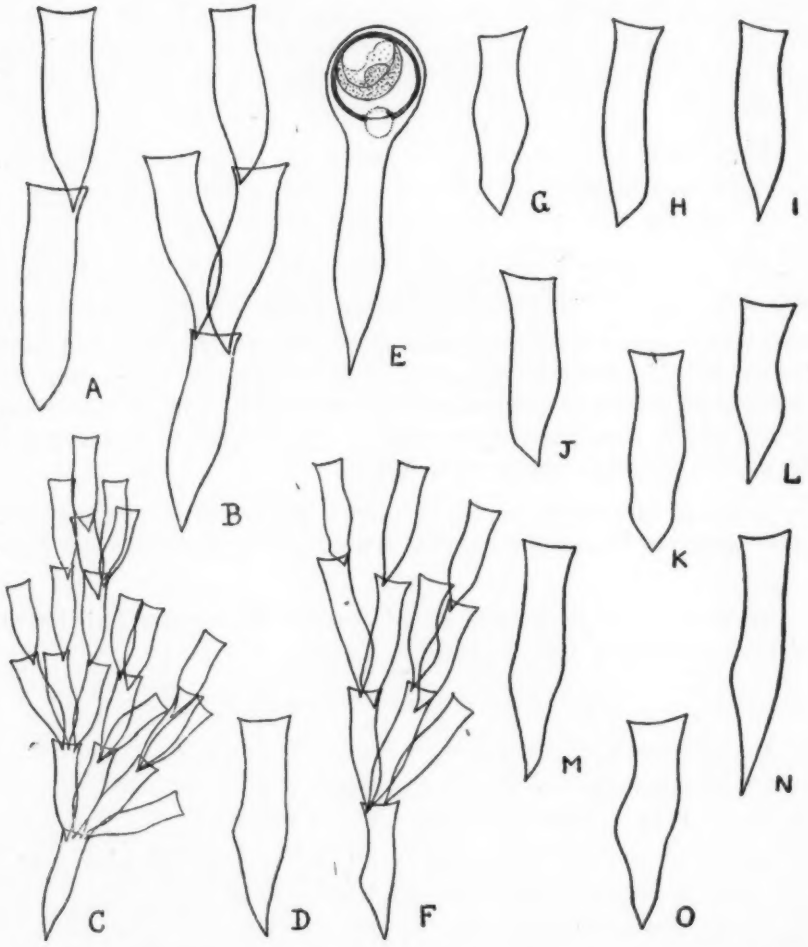
G. I. Playfair, 1921, p. 112, Pl. 1, Figs. 17–21;

G. Huber-Pestalozzi, 1941, p. 222, Fig. 290.

Colony densely bushy, lorica in the form of an asymmetrical vase with the portion below the orifice drawn out. Cysts not observed.

Dimensions of lorica.—

Length	.. .. ..	28·2–38·5 μ rarely up to 42·3 μ
Breadth at med. region	.. ..	7·9– 9·7 μ
„ below orifice	.. ..	6·2– 7·9 μ
„ at orifice	.. ..	8·8–10·6 μ



TEXT-FIG. 3.—*Dinobryon sertularia.* A, B, C, D, Typical form. A, B & D (×1,000), lorica from different regions of the colony. C (× 483), a colony; E–O, Form from Chandrakona. E (× 1,000), a lorica with cyst. F (× 587), a colony. G–O (×1,000); lorica from different regions of the colony.

The alga agrees fairly well with the type, European forms of which measure 30–44 $\mu \times 10$–14 μ and Australian and Javanese specimens 26–38 $\mu \times 7$–10 μ.

Hab.—Planktonic in large fish ponds of clear water at Kausalya Ganga, Orissa, during October-November 1952; in Rangamati " bundh ", Chandrakona, during December 1952; in Sim's Park pond, Coonoor, during June 1952; and in Periyar estuary, Azhicode, during October 1951.

Known distr.—Fairly widespread, in Europe, W. Greenland, Africa (L. Victoria) and Java (*cf.* Huber-Pestalozzi, 1941, p. 222), in N. America (G. M. Smith, 1933), p. 181, and in Australia (*cf.*, Playfair, 1921, p. 112).

Isolated specimens of a form of *D. sertularia* (Text-Fig. 3, E–O), which has slight protuberances from the base of some of the lorica, occurred in Rangamati " bundh " along with type specimens. The lorica has a length of 29·9–44 μ and a breadth of 7·9–8·8 μ in the median region, 6·2–7 μ below the orifice and 8·8–10·6 μ at the orifice. Cyst 14 μ in diameter. A variety of *D. sertularia*, *viz.*, var. *protuberans* (Lemm.) Krieger (*cf.* Huber-Pestalozzi, 1941, p. 223, Fig. 291), = *D. protuberans* Lemm. (*cf.* Lemmermann, 1910, p. 462, Pl. 29, Fig. 14), which is known from Europe, N. Asia and New Zealand, has distinct protuberances at the base of the lorica. The protuberances in the present form are not as pronounced as in this variety, and, it could not be ascertained whether it was an occasional variation of the type or whether it actually belonged to the variety *protuberans*.

9. *Dinobryon cylindricum* Imhof (Text-Fig. 4, A–O).

A. Pascher, 1913, p. 77, Fig. 123;

G. Huber-Pestalozzi, 1941, p. 223, Fig. 292.

Colony loosely bushy or spreading; lorica elongated, usually asymmetrical at the base and more or less cylindrical above, with a fairly wide orifice. Cysts not observed.

The alga was collected from two localities, *viz.*, (1) Ward Lake, Shillong (1·6 acres in area, max. depth 12 feet) during June 1951, and (2) Periyar estuary, Azhicode (freshwater area about one mile upstream) during October 1951. Shillong specimens (Text-Fig. 4, H–O) have their lorica 44–52·8 μ long, 7–10·6 μ broad and orifices 10·6–11·4 μ wide, whereas the Azhicode material (Text-Fig. 4, A–G) has lorica 42·3–48 μ long, 7–8·8 μ broad, and orifices 8·8–11 μ wide.

Known distr. including that of var. palustre *Lemmermann.*—Europe and Asia (*cf.* Huber-Pestalozzi, 1941, p. 224); N. America (*cf.* G. M. Smith, 1950, p. 424).

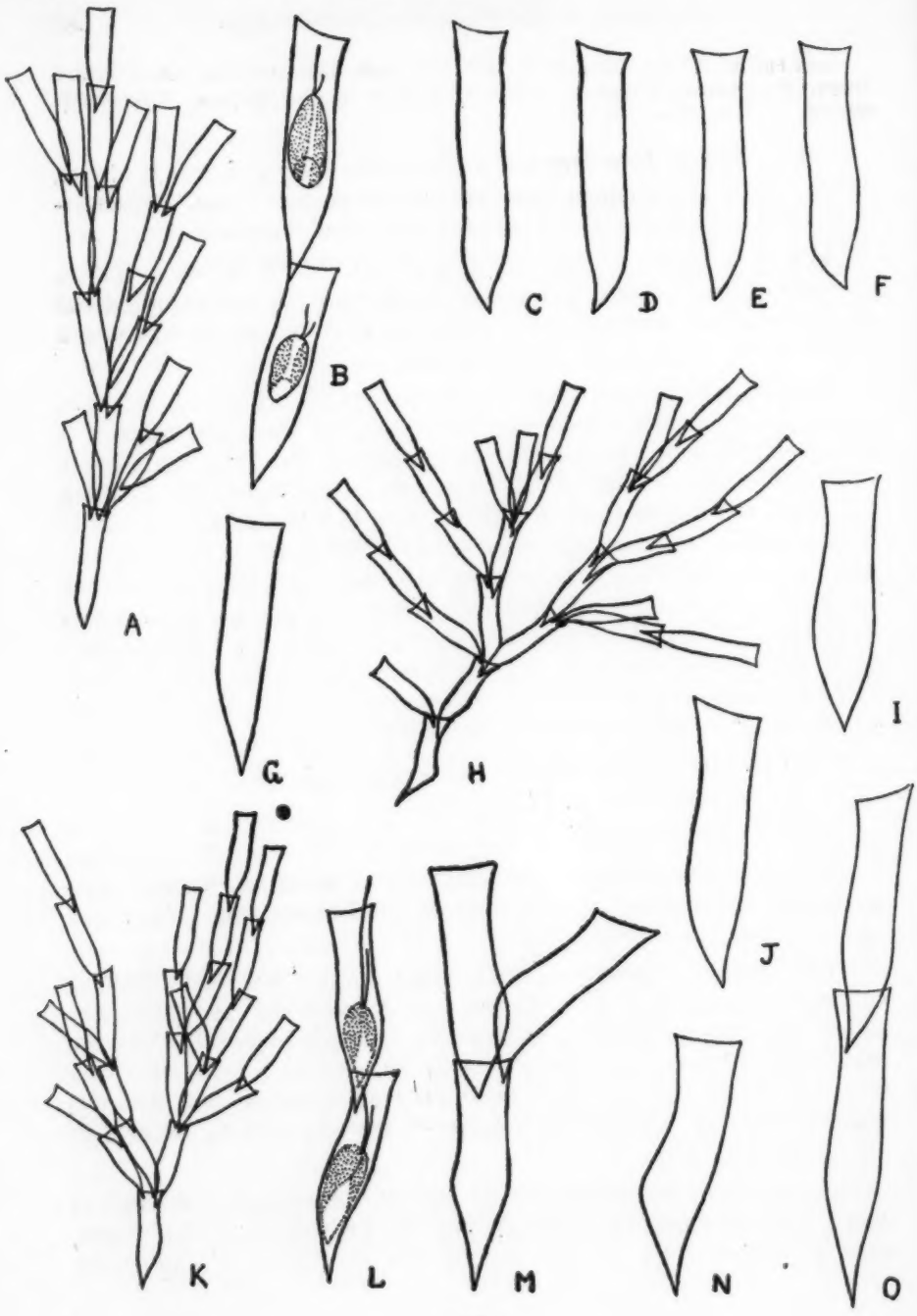


FIG. 4

TEXT-FIG. 4.—*Dinobryon cylindricum*. A-G, Material from Azhicode. A (× 483), a colony. B-G, Lorica enlarged (× 1,000); H-O, Material from Shillong. H, K (× 350), colonies. I-J, L-O, lorica enlarged. L (× 680), I-J, M-O (× 1,000).

10. *Dinobryon bavaricum* Imhof (Text-Fig. 5).

G. Huber-Pestalozzi, 1941, pp. 224–25, Fig. 295. Syn. *D. cylindricum* var. *ceylonicum* Lemm. 1907, p. 266, Figs. D–F.

Colony elongated and generally with a small number of lorica. Lorica with an elongated, straight or slightly curved, basal, stalk-like portion and an upper cylindrical portion having prominent undulations below and smaller undulations above. Cysts frequent.

Dimensions of lorica.—

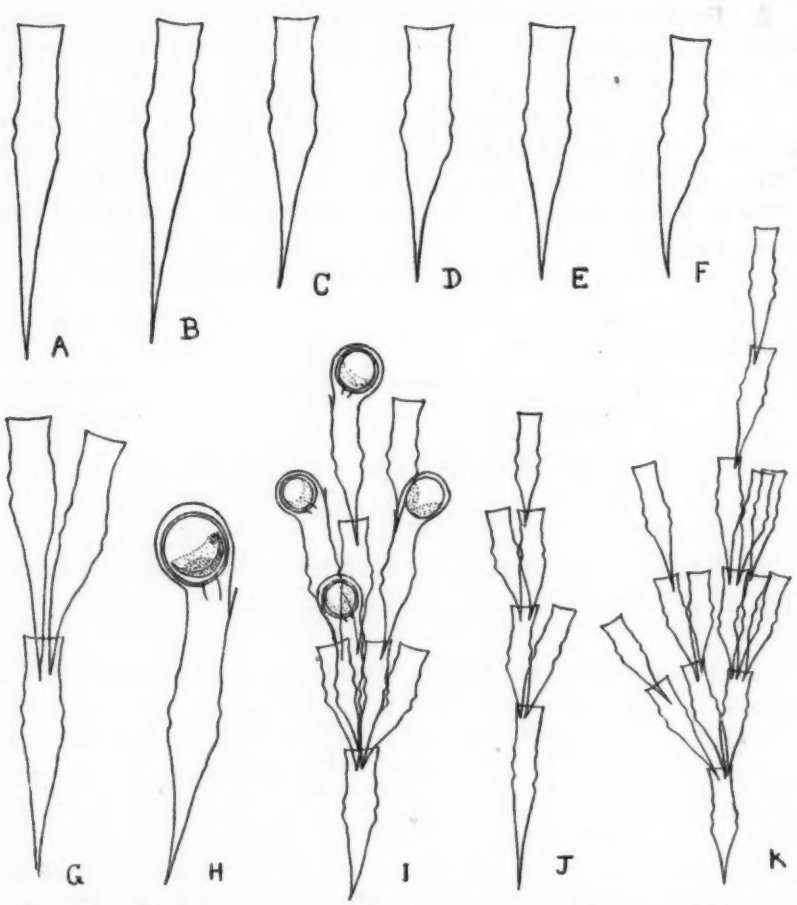
Length	37·0–56·3 μ
Breadth at medium region ..	7·9–8·8 μ rarely to 9·7 μ
„ below orifice	6·2–7·0 μ
„ at orifice	7·9–8·8 μ
Diameter of cyst..	10·6–11·4 μ

Hab.—Planktonic in large fish ponds of clear water in Kausalya Ganga, during October–November 1952; in fishery "bundhs" at Chandrakona during December 1952; and in a fish pond at Chaudwar, during January 1953.

Known distr.—Wide-spread in the plankton of northern moderate and cold zones (*cf.* Huber-Pestalozzi, 1941, p. 225); in N. America (*cf.* G. M. Smith, 1950, p. 424); and in Ceylon (*cf.* Lemmermann, 1907, p. 266).

In addition to *D. cylindricum* var. *ceylonicum* Lemm., Krieger (cited by Huber-Pestalozzi, *loc. cit.*) has included a large number of species and varieties [*D. stipitatum* Stein; *D. elongatum* Imhof; *D. stipitatum* var. *bavaricum* (Imhof) Zach.; *D. stipitatum* var. *undulatum* Lemm.; *D. elongatum* var. *affine* Lemm.; *D. bavaricum* var. *affine* Lemm.; *D. sociale* var. *bavaricum* (Imhof) Bachm.; *D. stipitatum* subspec. *eustipitatum* Pascher; *D. stipitatum* subspec. *bavaricum* Pascher; *D. stipitatum* var. *affine* (Lemm.) Pascher] to constitute this composite species. The present form is more or less identical with *D. cylindricum* var. *ceylonicum* Lemm., the only difference being that the lorica has a wider range of length (Lemmermann gives the length of 41–46·5 μ, whereas Huber-Pestalozzi gives 50–120 μ for the composite species *D. bavaricum*).

In the presence of a prominent median constriction and in the general shape of the *lorica*, the alga shows a superficial resemblance to *D. divergens* Imhof [*cf.* Huber-Pestalozzi, 1941, pp. 227–28, Fig. 302; = *D. cylindricum*



TEXT-FIG. 5.—*Dinobryon bavaricum.* A–G, Lorica enlarged (× 1,000). H (×1,000), Lorica showing cyst. I (× 587), Colony showing cysts. J & K (× 483), Colonies.

var. *divergens* (Imhof) Lemmermann, 1900, p. 517, Pl. 19, Figs. 15–20; Playfair, 1921, p. 113, Pl. 1, Figs. 24 and 25], but, the elongated small-sized colonies of the present alga, with the basal portion of its lorica drawn out into a stalk and the upper portion distinctly undulate, are more characteristic of *D. bavaricum* than of *D. divergens*.

SUMMARY

1. Ten flagellate species of Chrysophyceæ, four belonging to *Mallomonas* Perty, three to *Synura* Ehr., and three to *Dinobryon* Ehr. are described.

2. The localities from which these algæ were collected and the periods of their occurrence are given. They occur in the plains usually during winter and at high elevations during summer.

3. Except for one previous record of *Synura*, no flagellate members of the Chrysophyceæ appear to have been recorded in India so far. Records of even non-flagellate members of the class from India are few. The present records show that members of the Chrysophyceæ are not as rare in India as believed hitherto.

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ADDENDUM

After sending this paper to the press the author came across some of the species described above from the following localities as well:—*Mallomonas splendens* (isolated) from a canal at Chalakudi, Cochin, in February 1953; *M. Tonsurata* (isolated) from river Mahanadi near Cuttack during December 1952–January 1953; *Dinobryon sertularia* from Beauvoir fish pond, Mercara, Coorg (isolated), from river Mahanadi near Cuttack (isolated) and from a canal at Chalakudi, Cochin (isolated), all in February 1953. *Dinobryon cylindricum* resembling the Azhicode form, but with lorica 48·8–60 μ long, 6·5–7·5 μ broad and orifice 8–9·4 μ wide, was also observed (rare) in collections made from Aruvikara reservoir, Trivandrum, in February 1953.

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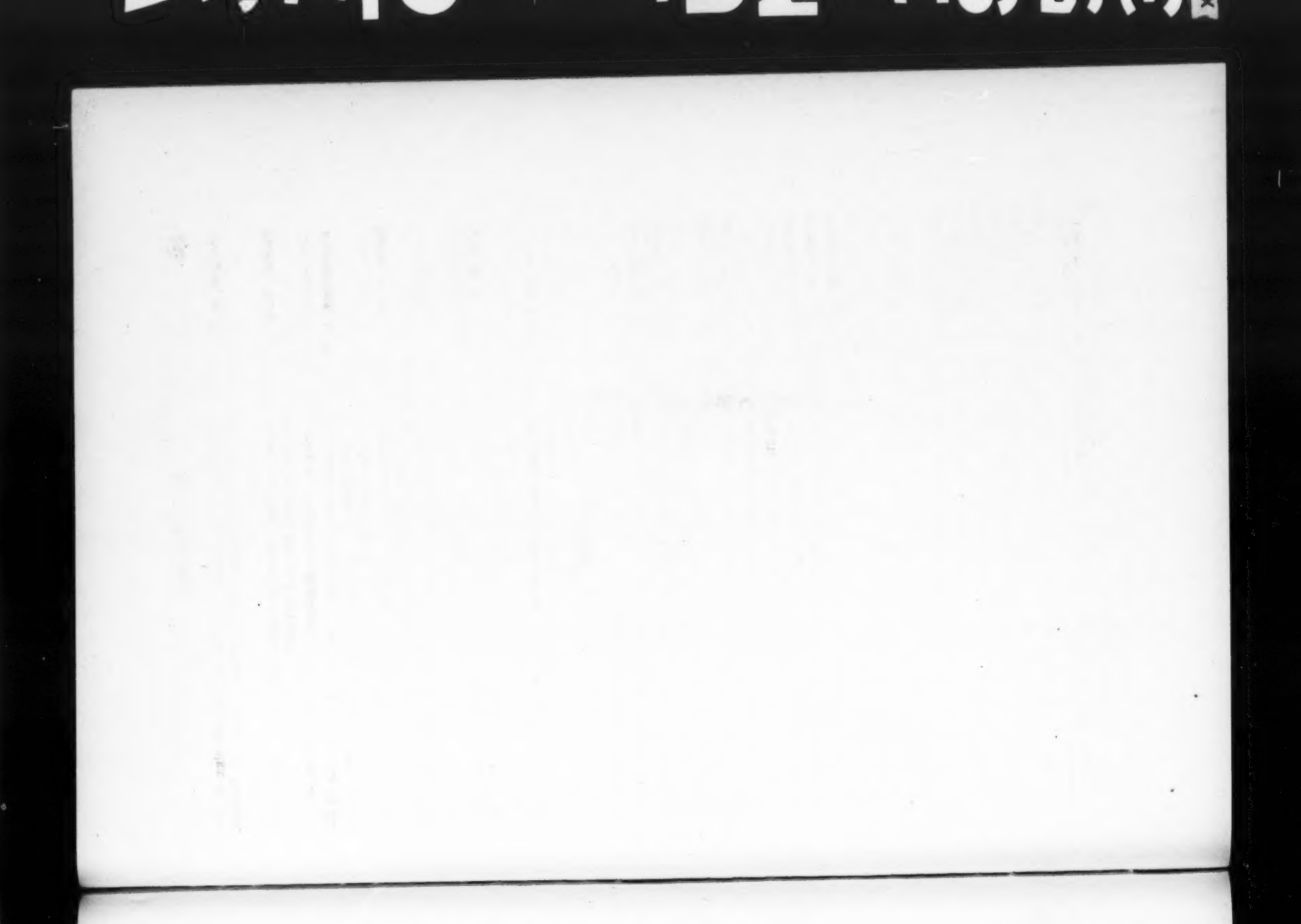

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CONTENTS

PAGE

Some Aspects of Adaptation in *Chanos chanos* (Forskål) . . . . . N. K. Panikkar, P. R. S. Tampi and R. Viswanathan 203

Epidemiological Aspects of the Bombay Duck or 'Bombil' (*Harpodon nehereus* Buch.) . . . . . M. J. Albuquerque and J. V. Bhat 214

Studies on Mackerel Fat Variations: Correlation of Plankton Fat with Fat of Fish . . . . . R. Venkataraman and S. T. Chari 224

On the Nature of the Spore-Appendage in *Neottiospora* Desm. . . . . . C. V. Subramanian and K. Ramakrishnan 228

Contributions to Our Knowledge of Indian Algæ. I. Chrysophyceæ—On the Occurrence of *Mallomonas* Perty, *Synura* Ehr., and *Dinobryon* Ehr. in India . . . . . M. T. Philipose 232

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INDEX TO VOL. XXXVII—(B)

AUTHORS' INDEX

Agnihothrudu, V. ..	Soil conditions and root diseases, VIII, 1.
Albuquerque, M. J. and Bhat, J. V.	Epidemiological aspects of the Bombay duck or 'Bombil' (*Harpodon nehereus* Buch.), 214.
Bharani, P. Smita, Shah, Y. S. and Sreenivasan, A.	Biosynthesis of vitamin C during germination, III, IV, 33, 54.
Bhat, J. V. ..	*See* Albuquerque and Bhat.
Bhatnagar, V. B. ..	*See* Choudhri and Bhatnagar.
Chari, S. T. ..	*See* Venkataraman and Chari.
Chatterjee, B. G. Ramamoorthy, B., Dakshinamurti, C. and Gulati, K. C.	Determination of nicotine in tobaccos, 22.
Choudhri, R. S. and Bhatnagar, V. B.	Prevention of premature bolting in onions following maleic hydrazide treatment, 14.
Cohly, M. A., Giri, K. V. and Rao, M. R. A.	A micro-electrophoretic study of human serum proteins in small-pox, 197.
Dakshinamurti, C. ..	*See* Chatterjee and others.
Giri, K. V. ..	*See* Cohly and others.
Gulati, K. C. ..	*See* Chatterjee and others.
Guttikar, M. N. ..	*See* Sohonie, Kamala and Guttikar.
Pai, K. V. Srinivasa ..	Carbon, nitrogen and pH relationships to growth of soil fusaria in culture, 131.
	See Subramanian and Pai.
Panikkar, N. K., Tampi, P. R. S. and Viswanathan, R.	Some aspects of adaptation in *Chanos chanos* (Forskål), 203.
Philipose, M. T. ..	Contributions to our knowledge of Indian algæ, I, 232.
Ramakrishnan, K. ..	*See* Subramanian and Ramakrishnan.
Ramakrishnan, T. S., Srinivasan, K. V. and Sundaram, N. V.	Additions to fungi of Madras, XIV, 83.
Ramamoorthy, B. ..	*See* Chatterjee and others.
Ramaswamy, A. S. and Rao, R. Rama	Experimental pneumokoniosis caused by beryl dust in guinea-pigs, 158.
Rao, L. S. Prahlada and Subramaniam, M. K.	Studies on the cytology of yeasts, VII, 72.

Rao, M. R. A. .. *See* Cohly and others.

Rao, R. Rama .. *See* Ramaswamy and Rao.

Sayeedud-Din, M. .. Observations on the anatomy of some of the convolvulaceæ, 106.

Sebastian, V. O. .. The development of *Herdmania pallider* (Heller), 174.

Shah, Y. S. .. *See* Bharani and others.

Singh, Inderjit .. Early tension relaxation during contraction of unstriated muscle, 114.

Singh, Inderjit and Singh, Sunita Inderjit — Role of the actomyosin in contraction of unstriated muscle, 188.

Singh, Sunita Inderjit .. *See* Singh and Singh

Sohonie, Kamala and Guttikar, M. N. — Stability of ascorbic acid in neera from date palm (*Phonix silverstres*), 167.

Sreenivasan, A. .. *See* Bharani and others.

Srinivasan, K. V. .. *See* Ramakrishnan and others.

Subramaniam, M. K. .. Abnormal mitoses in tetraploid yeasts, 27.
See Rao and Subramaniam.

Subramanian, C. V. .. Fungi imperfecti from Madras, IV, 96.

Subramanian, C. V. and Pai, K. V. Srinivasa — Relation of nitrogen to growth and sporulation of *Fusarium vasinfectum* Atk., 149.

Subramanian, C. V. and Ramakrishnan, K. — *Petrakomyces*, a new genus of the sphæropsidales, 110.
On the nature of the spore-appendage in *Neottiospora* Desm., 228.

Sundaram, N. V. .. *See* Ramakrishnan and others.

Tampi, P. R. S. .. *See* Panikkar and others.

Venkataraman, R. and Chari, S. T. — Studies on Mackerel fat variations: correlation of plankton fat with fat of fish, 224.

Viswanathan, R. .. *See* Panikkar and others.

TITLE INDEX

Algæ, Indian, contributions to our knowledge, I (Philipose), 232.

Ascorbic acid, stability, in neera from date palm (*Phonix silverstres*) (Kamala Sohonie and Guttikar), 167.

Bombay duck or 'Bombil' (*Harpodon nehereus* Buch.), epidemiological aspects (Albuquerque and Bhat), 214.

Chanos chanos (Forskål), some aspects of adaptation (Panikkar and others), 203.

Convolvulaceæ, some, anatomy, observations (Sayeedud-Din), 106.

Fungi imperfecti from Madras, IV (Subramanian), 96.

Fungi of Madras, additions, XIV (Ramakrishnan and others), 83.

Fusarium vasinfectum Atk., growth and sporulation, relation of nitrogen (Subramanian and Pai), 149.

Herdmania pallida (Heller), the development (Sebastian), 174.

Human serum proteins in small-pox, a micro-electrophoretic study (Cohly and others), 197.

Mackerel fat variations, studies: correlation of plankton fat with fat of fish (Venkataraman and Chari), 224.

Muscle, unstriated, contraction, role of actomyosin (Singh and Singh), 188.

Muscle, unstriated, early tension relaxation during contraction (Singh), 114.

Neottiospora Desm., spore-appendage, on the nature (Subramanian and Ramakrishnan), 228.

Nicotine in tobaccos, determination (Chatterjee and others), 22.

Onions, prevention of premature bolting, following maleic hydroxide treatment (Choudhri and Bhatnagar), 14.

Petrakomyces, a new genus of the sphæropsidales (Subramanian and Ramakrishnan), 110.

Pneumokoniosis, experimental, caused by beryl dust in guinea-pigs (Ramaswamy and Rao), 158.

Soil conditions and root diseases, VIII (Agnihothrudu), 1.

Soil fusaria, growth, in culture, carbon, nitrogen and pH relationships (Pai), 131.

Vitamin C, biosynthesis, during germination, III, IV (Bharani and others), 33, 54.

Yeasts, cytology, studies, VII (Rao and Subramaniam), 72.

Yeasts, tetraploid, abnormal mitoses (Subramaniam), 27.